



Europäisches Patentamt

European Patent Office

Office européen des brevets

(19)

(11) Publication number:

0 104 702

A1

(12)

## EUROPEAN PATENT APPLICATION

(21) Application number: 83201335.3

(51) Int. Cl.<sup>3</sup>: A 01 K 61/00

(22) Date of filing: 15.09.83

A 23 K 1/18

(30) Priority: 24.09.82 NL 8203710

(72) Inventor: Sorgeloos, Patrick P.  
No. 7, Tweekoningenstraat  
B-9800 Duffel(BE)

(43) Date of publication of application:  
04.04.84 Bulletin 84/14

(72) Inventor: Verschaele, Danny H.  
No. 95, Lokerenbaan  
B-9140 Zele(BE)

(84) Designated Contracting States:  
AT BE CH DE FR GB IT LI LU NL SE

(72) Inventor: Leger, Philippe P.J.M.  
No. 31, Bakkerstraat  
B-8870 Wervik(BE)

(71) Applicant: Artemia N.V.  
Anneessensstraat 1-3 P.O. Box 1  
B-2000 Antwerpen(BE)

(72) Inventor: Lavens, Patrick A.  
No. 90, Ruddervoordestraat  
B-8100 Torhout(BE)

(54) Method and device for producing, packing, and using artemia offspring.  
(57) Artemia (14,90) are kept under such conditions in high density in a flowing aqua-culture (2) that either nauplii (90) or cysts (14) are deposited as offspring (14,90).  
A treatment inducing a cyclic cysts production consists of a cyclic reduction of the oxygen concentration of the flowing aqua-culture (2).  
A device (1,44) for depositing only one selected type of offspring (14,90) comprises for the production of cysts (14) inducing means (12,53,64,67) for the cyclic reduction of the oxygen concentration.

A packing (67) of such offspring (14,90) is provided with a label (68) indicating a specification predetermined by the conditions the flowing aqua-culture (2) had been operated with.

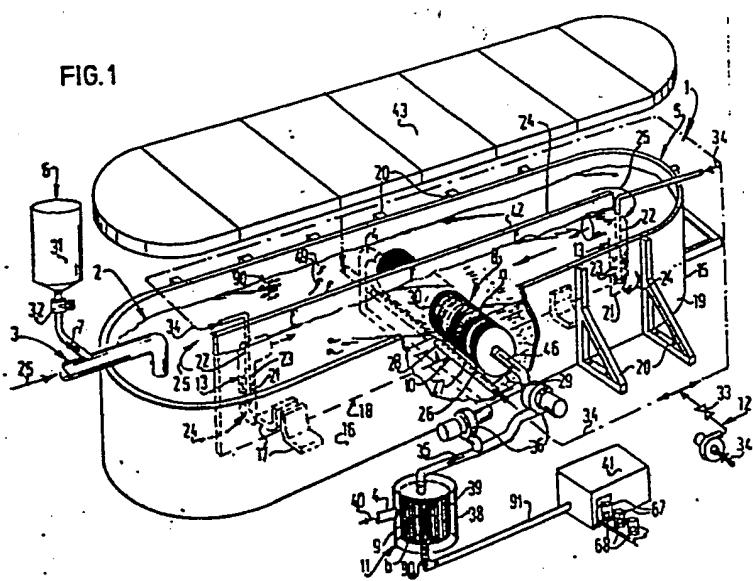
Methods for feeding Artemia (14,90) are characterized by the use of agricultural derivatives (7) and/or chicken manure (7).

A method for preparing food (14,90) is characterized by the use of nauplii (90) and/or cysts (14) produced in the flowing aquaculture (2) and/or by the use of particles coated with a food additive.

EP 0 104 702 A1

1...

**FIG. 1**



## TITLE &amp; SUBTITLE

see back page

-1-

Method and device of producing Artemia offspring; packing of Artemia offspring; method of feeding Artemia; method of preparing food for larva of fish or crustacea; food comprising particles coated with a food additive and method of 5 producing animal food.

The invention relates to a method of producing Artemia offspring in which the reproducing Artemia are kept in aquaculture and the offspring is harvested.

10 This method is known and as far as the production of cysts is concerned it is described in the article "Controlled production of Artemia cysts in batch cultures" by D. Versichele and P. Sorgeloos in the book "Brine Shrimp Artemia", Vol. 3, "Ecology culturing use in aqua-culture", 15 authors: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers, page 231 to 246, Universa Press, Wetteren, Belgium.

Artemia, the brine shrimp, is found restrictively in salt lakes throughout the world. Due to the extreme ecologic conditions in these biotopes, for example, a salinity of more 20 than 100 gs per litre of water, predators and food competitors of Artemia are lacking and the Artemia can flourish in these biotopes. As a result of varying environmental conditions, for example, drying up of the salt lake, the manner of reproduction changes in a sense such that the reproduction changes 25 from an ovoviviparous reproduction (living offspring) into an oviparous reproduction (inactive offspring). As living offspring nauplii are produced which grow up to adult Artemia within 9 days to 6 weeks (dependent on the culture conditions). In the case of the oviparous reproduction the embryonic 30 development is reversibly discontinued during the gastrulation.

Each gastrula is surrounded by a sheel impregnated with haematin-like substance. These encapsulated gastrulae are termed cysts, which are excreted in the water. The cysts remain floating in the salt water and subsequently are 5 washed on the shore. After processing, the cysts can be dried or kept under anaerobic conditions for many years. After the cysts are again placed in sea water the metabolism of the embryo is activated and after a few hours a free-swimming nauplius is set free of the cyst.

10 Artemia nauplii are used as food for the larvae of fish and crustacea. Because Artemia cysts can be kept for a long time and free-swimming nauplii become available a few hours after introduction of the cysts into salt water by expansion of the cysts Artemia cysts constitute an ideal source 15 of food for larvae of fish or crustacea.

However, the production of Artemia offspring in nature is too low to meet the need. Apart therefrom Artemia offspring produced in nature have the disadvantage that they may be contaminated by any kind of toxic compound, for example 20 chlorinated hydrocarbons.

Scientific tests revealed that the batch culture production of one type of offspring on a technical scale is not feasible, since the batch culture production of Artemia offspring has the drawback that due to pollution, for example, 25 by faeces, metabolites, peeling and bacteria the water quality is deteriorated so that the batch culture has to be refreshed each week, even in the case in which the offspring producing Artemia is kept at low density in the batch culture. However, a low density of Artemia results in a low production 30 per cubic metre and a low degree of usefulness of the food added to the batch culture. Due to the necessarily low density of Artemia in a batch culture the volume in which the Artemia are swimming is smaller than the overall volume of the batch culture, which means that only part of the 35 total volume is populated by the Artemia. On the one hand this means that the food present in the unpopulated volume, remains unused (the degree of use of the available food is, therefore, low) and on the other hand it means that the quan-

tity of food in the populated volume has to meet the daily need.

The invention has for its object to provide an improved method which avoids the disadvantages inherent in the use of 5 batch culture by keeping the Artemia in high density in a flowing aqua-culture. The density is preferably of the order of magnitude of at least 1000 animals per litre.

In the flowing aqua-culture the water has a residence time of, for example, about one hour. This period of time is 10 so short that the excretion produced by the Artemia only slightly affects the water quality in the aqua-culture. This permits of increasing the density of the Artemia in the aqua-culture, whilst at the same time the degree of use of the good is raised.

15 The amount of fresh water to be supplied per hour to the aqua-culture can be reduced by purifying and subsequently recycling at least part of the water conducted away as a carrier of offspring and excreta after harvesting the off-spring produced.

20 If cysts are produced in the flowing aqua-culture, the Artemia are subjected, in order to the production of Artemia cysts, to a process inducing a cyclic cyst production, preferably by decreasing the oxygen concentration in the flowing aqua-culture as a process inducing cyst products.

25 When used in a batch culture this method has the disadvantage that it takes a very long time (ten minutes) before, after stopping the supply of air to the batch culture, the oxygen concentration has become so low that a shift towards an oviparous reproduction is brought about. Moreover, it takes 30 long time before, after restart of the air supply the oxygen concentration has attained the optimum value so that due to this long period of lack of oxygen high mortality occurs.

With the higher density of Artemia the need of oxygen in the flowing aqua-culture increases. This means that after 35 stopping the air supply through the aerating means the oxygen concentration in the aqua-culture drops more rapidly so that within a shorter time a lower oxygen concentration is attained. As a result the stress conditions become more

extreme. Since the Artemia are thus stimulated earlier to oviparous reproduction, it is possible to reduce the magnitude of another stress-determining factor, preferably by decreasing the conventional salinity of the water in the aqua-culture 5 of more than 90 gs of salt per litre of water (ppt). Preferably the aqua-culture is flushed with water, the salinity of which lies between 15 and 90 ppt and more; preferably the aqua-culture is flushed with sea water.

In the case in which for reducing the oxygen concentration 10 in the aqua-culture the air supply is stopped at the aerating means, the water circulation produced by the gas bubbles formed by the aerating means will decrease. This may give rise to clogging of the sieve means, as a result of which the aqua-culture spills over and loss of animals 15 occurs.

During the cyclic reduction of the oxygen concentration clogging and overflow of the aqua-culture can be avoided by maintaining the vertical water circulation with the aid of the gas bubbles generated by the aerating means. This is 20 preferably achieved by reducing the oxygen concentration by supplying nitrogen gas to the aqua-culture.

By reducing the salinity of the water the specific weight of the water decreases and becomes equal to the specific weight of the produced cysts. The produced cysts will no 25 longer float on the water, but remain in suspension owing to the strong water circulation so that the produced cysts are preferably harvested from the stream of water leaving the aqua-culture.

The invention furthermore relates to and provides a 30 device for producing Artemia offspring in aqua-culture comprising a water basin, supplementary food means, sieve means for removing excreta and produced offspring from the aqua-culture, whilst retaining the Artemia producing offspring, aerating means and harvesting means and is characterized in 35 that the device is provided with means ensuring the deposition of only one selected type of the two potential types of offspring, i.e. nauplii and cysts and in that the water basin . . . . . with a water inlet and a water outlet for

producing a flowing aqua-culture.

The invention furthermore relates to a method of feeding Artemia in which the Artemia are fed by a micronized product.

5 This method is known. Artemia are obligatory "particle feeders" and in nature they feed on algae and bacteria. On an industrial scale Artemia are fed on micronized products such as dried blue sea-weeds, yeast, wheat-, fish- or rice-meal, egg cholk and homogenized liver. However, these products  
10 are expensive. In Southeast Asia manually ground rice chaff is used instead of the above-mentioned products, which is a waste product of the treatment of rice and hence cheap. Rice chaff is hardly usable in Europe as a food for feeding Artemia on an industrial scale because micronizing rice chaff brings  
15 about high costs. Apart therefrom the rice chaff available in Europe is of highly varying qualities and may be contaminated with pesticides.

The invention has for its object to provide a micronized product improved in this respect since the Artemia are fed on  
20 a carbohydrate- or protein-lean maize derivative, a soya derivative and/or a manioc derivative.

From an economic point of view the use of these derivatives is interesting because they are largely available and because, for example, the maize derivative is a waste product  
25 of the maize treatment, for example, maize stem parts, mechanically worn-off parts of the grains and/or dust of grain strains the maize derivative is cheap. Furthermore the use of the maize derivative has the practical advantage that, for example, in contrast to rice chaff, it has a better food  
30 conversion which means that the nutrient substances contained in the maize derivative are more readily available so that as compared with rice chaff a lower amount of maize derivative is required. Finally the maize derivative contains less soluble substances so that pollution of the aqua-culture is reduced  
35 and the risk of clogging of the strainer is lowered and the water emanating from the aqua-culture need be purified to a lesser extent.

The use of the carbohydrate- and/or protein-lean maize

derivative in itself is remarkable because the maize derivative does not satisfy the conditions mentioned in literature for Artemia food. Nevertheless it has been found that the carbohydrate- and/or protein-lean maize derivative has a high nutritional value for the Artemia.

The invention also relates to a different method of feeding Artemia, in which the Artemia are fed on a micronized product and which is characterized in that the Artemia are fed on micronized chicken manure. Micronized chicken manure is 10 an excellent food for Artemia. Owing to the short resident time of food in the gastric-intestinal canal of the chicken manure still contains a high concentration of nutritional substances. Moreover, chicken manure has a high food conversion value and it contains adequate particulate carrier material. 15 Like the other derivatives chicken manure is cheap and largely available.

The invention furthermore relates to a method of preparing food for larvae of fish crustacea in which nauplii released by the development of Artemia cysts or obtained by 20 ovoviparous reproduction are fed to the larvae and which is characterized in that Artemia cysts or nauplii produced in a flowing aqua-culture are employed.

Since in contrast to nature the Artemia in aquaculture are fed on terrestrial food (rice chaff, maize derivatives and the like), the nutritional value of the nauplii directly or indirectly obtained through cysts will not satisfy the food need of marine animals. The nutritional value of nauplii can be adjusted to the food need of larvae by adding preferably such food additives to the aqua-culture that the 30 food composition of the nauplii corresponds to the food need of the larvae.

The invention furthermore relates to a method of adjusting the food composition of nauplii obtained by Artemia and serving as food for larvae of fish or crustacea to the food 35 need of the larvae characterized in that at least one food additive in the form of particle coatings is added to the Artemia. The food additive, preferably fish oil and more

preferably codliver oil can be added in the form of a coating on particles, preferably rice, starch grains having a suitable particle-size distribution and satisfactory coating properties to Artemia depositing offspring. It is furthermore 5 preferred to use particles of food intended for consumption by Artemia. In this case the carrier of the coating represents the basic food.

The above-mentioned and further features will be described more fully with reference to the drawing of a few 10 non-limitative examples.

The drawing schematically shows in

Figs. 1 and 2 each a perspective, fragmentary view of different devices for producing Artemia offspring in flowing aqua-culture, nauplii being produced in the device of Fig. 1 15 and cysts in the device of Fig. 2;

Fig. 3 a schematic representation as a function of time of an oxygen concentration reduction inducing deposition of cysts in the aqua-culture and

Fig. 4 an enlarged sectional view taken on the line 20 IV-IV in Fig. 2.

Fig. 1 shows a device 1 embodying the invention for producing Artemia nauplii 90 in a flowing aqua-culture 2. The device 1 comprises a water basin 5 having a water inlet 3 and a water outlet 4, food supplementing means 6 for adding 25 food 7 to the aqua-culture 2, sieve means for conducting away waste and produced nauplii 90 via the water outlet 4 whilst retaining the Artemia 49, aerating means 10, water circulating means 13, harvesting means 11 for harvesting the produced nauplii 90 and depositing means 12 ensuring that 30 the Artemia 49 deposit only one selected type of offspring, in this case nauplii 90, of the two potential types of offspring 14, 90 i.e. cysts 14 and nauplii 90.

The water basin 5 mainly has the shape of an endless raceway and comprises a wall 15, a bottom 16 and a central 35 wall 42, which is connected with the bottom 16 through supports 17 in a manner such that the lower longitudinal surface 18 of the wall 42 is free of the bottom 16. Along the circumference of the wall 15 struts 20 are arranged on the

outer surface 19 of the wall 15.

The water circulating means 13, for example, an "air water lift" comprise two substantially knee-shaped pipes 21 and air inlets 22 extending down in said pipes 21. The air 34 brought through each air inlet 22 towards the lower end of the associated pipe 21 rises in the form of gas bubbles 23 carrying along water 24 in the pipe 21, after which the water 24 flows in a horizontal direction out of said pipe 21. The pipes 21 are relatively disposed in the device embodying the invention in a manner such that a circulating stream 25 is generated in the basin 5.

The food supplementing means 6 comprise a container 31 for food 7. The food 7 is dosed by dosing means 32 through the water inlet 3 and supplied to the water 25 in the basin 5. 15 The food 7 preferably consists of micronized carbohydrate-and/or protein-lean maize derivatives of chicken manure.

Referring to Fig. 1, the deposition means 12 comprise a valve 33 by which the supply of air 34 to the water circulating means 13 and the water aerating means 10 can be 20 regulated so that the conditions in the aqua-culture 2 for the Artemia 49 are at an optimum for depositing nauplii 90.

The sieve means 8 disposed in the water basin 5 preferably comprise a wedge-shaped strainer 26. The embodiment of Fig. 1 shows a cylindrical, wedge-shaped strainer 26. Below 25 the wedge-shaped strainer 26 are arranged the aerating means 10 comprising a pipe 28 having holes 27 in a manner such that gas bubbles 29 produced by the aerating means 10 flow along slots 30 of the wedge-shaped strainer so that clogging of the strainer slots 30 is avoided.

30 The slot width a of the wedge strainer is such that whilst the Artemia 49 are retained, the water 35 containing the nauplii 90 and excreta 9 is removed from the aqua-culture through the water outlet 4. Through pumps 36 and duct 37 the water 35 is conveyed towards the harvesting means 11.

35 The harvesting means 11 comprise a cylindrical wedge strainer 39 immersed in a container 38. The wedge strainer 39 has a slot width b varying between 100 and 200  $\mu$ ms. The wedge-strainer slot width b is such that water 40 containing excreta

flows through the wedge strainer 39 and is evacuated through the outlet 4 of the container 38. The nauplii 90 sieved by means of the wedge strainer 39 from the excreta containing water 35 are conveyed through a duct 91 for processing towards 5 a processing unit 41.

Fig. 2 shows a different device 44 embodying the invention in which the water inlet 3, the water basin 5 and the water outlet 4 are included in a recycling circulation. In this device 44 Artemia cysts 14 are produced. The deposition means 12 comprise for this purpose induction means by which the Artemia 49 producing the cysts 14 are subjected to treatment inducing a cyclic cyst production. In the case of Fig. 2 the treatment inducing the cyclic cyst production consists of the cyclic reduction of the oxygen concentration 15 in the flowing aqua-culture 2. The induction means 12 comprise a control-unit 53 by which air 34 and nitrogen gas 64 are alternately supplied through ducts 83 and 54 respectively to the air inlet 22 in the water circulating means 13 and the pipe 28 with holes 27 in the water aerating means 10 to the 20 aqua-culture 2. During the period of nitrogen gas supply to the aqua-culture the oxygen concentration in the aqua-culture 2 drops to an extent such that a deposition of cysts 14 is induced. In the device 44 the oxygen concentration in the aqua-culture 2 is reduced more rapidly because during the 25 period in which nitrogen gas 64 is supplied to the aqua-culture 2 through the water circulating means 13 and the aerating means 10 oxygen-lean water 56 is supplied to the aqua-culture 2 through the water inlet 3. The water 56 is oxygen-depleted in a gas exchange unit 57. This unit 57 is 30 provided on the underside with two inlets 58 and 59. Through the inlet 59 water 60 is supplied to the unit 57. Above in the unit 57 the water passes along an overflow 61 and is then supplied through a duct 62 to the water inlet 3. During the period in which through the control-unit 53 nitrogen gas 64 35 is supplied through the ducts 83 and 54 to the aqua-culture 2 nitrogen gas 64 is also supplied through the duct 65 by way of the inlet 58 to the gas exchange unit 57. In the gas exchange unit 57 the nitrogen gas dissolved in the water 60

is replaced by the nitrogen gas 64 so that through the duct 62 oxygen-lean, nitrogen-gas enriched water 56 is supplied to the aqua-culture 2 and oxygen-enriched nitrogen gas is conducted away from the gas exchange unit 57 through the 5 vent 66.

The nitrogen gas 64 required for the cyclic reduction of the oxygen concentration in the flowing aqua-culture 2 originates from a container 84 containing the nitrogen gas 64.

As the case may be, the oxygen concentration in the 10 aqua-culture 2 may be reduced by interrupting the supply of air 34 to at least part of the aerating means 10 and/or the water circulating means 13.

Whilst Artemia 49 are retained, water 46 containing cysts 14 and excreta 9 is removed through the wedge strainer 15 26 from the aqua-culture and conveyed through duct 47 to the harvesting means 11. After the removal of the cysts 14 particle-sized excreta is sieved out of the water 46 in a strainer unit 48, the water 46 is purified in a biological purifying unit 50 from organic refuse and in a desinfection unit 51 20 the water 46 is desinfected after which the purified water 52 is supplied through the inlet 3 to the aqua-culture 2 and the recirculation 45 is closed. The excreta from the strainer unit 48 and from the biological purifying unit 50 is conducted away 25 through the duct 82. Through the water inlet 3 an amount of fresh water is constantly supplemented as is required for maintaining a constant amount of water 25 in the device 44.

In nature the Artemia are incited by varying environmental conditions to produce cysts. Under artificial conditions the Artemia can be stimulated to produce cysts by subjecting 30 the Artemia, in accordance with the invention, to a treatment inducing a cyclic cyst production. This treatment may consist of a cyclic reduction of the oxygen concentration, which can be performed by cyclically discontinuing the supply of air to the aqua-culture, by supplying nitrogen instead of air to the 35 aqua-culture and/or by adding substances to the aqua-culture which reduce the oxygen concentration or convert the oxygen contained in the aqua-culture into a form unusable for the

cysts by adding substances inducing the cyst production to the aqua-culture. Scientific research has shown that the change-over from ovovivi- to oviparous reproduction is induced by stress conditions which stimulate the haemoglobin production  
5 (under stress conditions the Artemia become red as a result of the increase in haemoglobin concentration, which compound has a red colour). The deposition of cysts may, if desired, be increased by adding to the aqua-culture iron which is an essential metal for haemoglobin production as a complex, for  
10 example, Fe-edta. Under artificial conditions the stress conditions stimulating the haemoglobin production may be imitated by applying an oxygen stress consisting of the cyclic reduction of the oxygen concentration and, moreover, by raising the salinity in the aqua-culture to at least 90 ppt.

15 Since the water in the flowing aqua-culture has a residence time of one hour, the excreta produced by the Artemia producing offspring will affect the water quality only to a minor extent. It is now possible to raise the density in the flowing aqua-culture to a high density, for  
20 example, of preferably at least 1000 animals per litre. With a density of preferably at least 10.000 animals per litre Artemia offspring can be produced in an economically advantageous manner, whilst with a density of the order of magnitude of preferably 15.000 animals per litre the production is at  
25 an optimum. The high density of Artemia results on the one hand in a higher offspring production per cubic metre water and on the other hand an increase in need for oxygen per cubic metre of water. In the case of cyst production this means that during the oxygen stress in the period in which  
30 instead of air nitrogen gas is supplied to the aqua-culture the oxygen concentration will drop more rapidly and to a lower value owing to the higher need for oxygen. Consequently water can now be used, the salinity of which is lower, preferably a salinity of 5 to 180 ppt, whilst it is now also possible  
35 to pass natural sea water through the aqua-culture (salinity 35 ppt).

Fig. 3 illustrates the decrease in oxygen concentration in the flowing aqua-culture after at the time 0 minutes

nitrogen gas is supplied to the aqua-culture rather than air. The lines c, d, e indicate the oxygen concentration as a function of time, when nitrogen gas is supplied for 3, 4 and 5 minutes respectively. From Fig. 3 it is apparent that the minimum oxygen concentration of 10% oxygen saturation to be preferred is obtained when nitrogen gas is supplied for at least 4 minutes. It is furthermore apparent that the whole stress period (oxygen deficiency) is at a maximum 20 to 25 minutes. This period is so short that an increased mortality of the population due to oxygen deficiency does not or only hardly occur. The line f indicates that, when the flowing aqua-culture is not covered, the minimum oxygen concentration is attained only after 13 minutes as a result of the transport by means of oxygen diffusion from the open air to the aqua-culture, whilst constantly supplying nitrogen gas. As a result of the flow in the aqua-culture an optimum oxygen concentration of 60% saturation will be attained within a short time after the nitrogen supply is stopped and air is again supplied, as compared with a batch aqua-culture.

The initial production of cyst is preferably raised by starting the aqua-culture with Artemia not yet sexually mature and by exposing these not yet adult animals to stress conditions, that is to say, reduction of oxygen concentration. In this way the Artemia are prevented from producing nauplii at the first deposition of living offspring.

The production of Artemia offspring per cubic metre may be further enhanced by using, by preference, partenogenetic Artemia strains. Such strains have the advantage that the population is only formed by females so that as compared with a bisexual strain the yield per cubic metre is about twice as high. As a partenogenetic strain for example, the Lavalduc strain may be employed.

In the method of producing Artemia offspring in accordance with the invention it is possible to produce Artemia offspring for at least three to four months with the same animals.

In the processing unit 41 (Figs. 1 and 2) the offspring 14, 90 is processed in a manner known per se and stored

in packings 67. For the cysts 14 the process consists in separating out impurities such as faeces, empty cyst shells and skins by means of density fractionation with stimulating solutions. The purified cysts are washed with water, dried 5 and packed under nitrogen or in vacuo in packings 67.

Fig. 4 shows the substantially cylindrical wedge 26 formed by rings 72 of wedge-shaped section arranged between two circular plates 70 and 71. Between the rings 72 is formed a wedge strainer slot 30 of accurately defined width a. The 10 aerating means 10 comprise a pipe 75 having holes 74. The pipe 75 is arranged below the wedge strainer 26 in a manner such that gas bubbles emanating from the holes 74 flow across a sieving surface 76 along the wedge strainer slots 30. In this way a turbulence is produced in the wedge strainer slots 15 30 so that clogging of the wedge strainer slots 30 by excreta 9 is avoided.

The wall 9 comprises an aluminium outer layer 77, an inner layer 78 of PVC foil and an insulating layer 79 sandwiched between the two layers 77 and 78. The insulating 20 layer 79 covers the whole outer side of the water basin 5. Heating means 80 are arranged below the bottom 16 of the water basin 5.

Among the offspring produced the nauplii are directly and the cysts are indirectly suitable as a food for larvae 25 of fish or curstacea. The cysts provide, in addition, the possibility of satisfying substantially instantaneously a need for very small living food. By introducing the Artemia cysts into salt water, the cysts develop and within a few hours living nauplii become available. The offspring produced 30 by the method embodying the invention have the advantage that they are produced under controlled conditions so that, in contrast to the offspring produced in nature, no unexpected contamination with toxic substances such as chlorinated hydrocarbons and heavy metals can occur, which may be toxic by the 35 directly produced nauplii or the nauplii developed from the cysts including toxic substances to the larvae so that the whole fish culture would be destructed. Moreover, by the method embodying the invention the offspring to be produced

may be adjusted in size to specific larvae of fish or crustacea by selecting an Artemia strain in which the size of the nauplii is smaller than the size of the alimentary mouth of the larvae.

5 It is preferred to adjust not only the biometric size of the nauplii but also the food composition to the alimentary need of the larvae in order to obtain optimum growth of the larvae. For example, a Brasilian Artemia strain may be suitable for the larvae with respect to biometry, but for an 10 optimum growth the cysts produced must have, for example, the lipide composition of a Chinese Artemia strain. Since the food composition is mainly determined by the food and the environment in which the Artemia producing offspring are kept and not genetically determined, it is preferred in the method 15 embodying the invention to add such food additives to the aqua-culture that the food composition of the nauplii corresponds to the food need of the larvae. For example, with a concentration of the fatty acid 20 : 5ω3 (a fatty acid of 20 carbon atoms and from the third carbon atom 5 conjugated 20 double bonds) by adding a high concentration of this food additive containing lipid, for example, blood to the aqua-culture the concentration of this lipid in the nauplii can be raised and be thus adjusted to the need of the larvae. If 25 the larvae are intended to serve as a food for sole it is preferred to add carotenoids to the aqua-culture so that with respect to the concentration of carotenoids the food composition is improved, as a result of which pigmentation of the skin of the sole is improved.

In a further method embodying the invention the food 30 composition of nauplii obtained from Artemia as a food for larvae of fish or crustacea can be adjusted to the food need of the larvae by adding at least one food additive in the form of particles coated with the food additive to the Artemia obligatorily feeding on particles. Since the Artemia 35 are fed by food originating directly (maize derivative) or indirectly (chicken manure) from terrestric (land) plants and since this terrestric food, for example, with respect to fatty acid pattern, deviates from that of marine food, the

food composition of the Artemia offspring is incomplete and the growth of the larvae is delayed. By additionally feeding the Artemia eating obligatorily particles with particles coated with at least one food additive, said particle in 5 itself having poor nutritive value, it is possible to selectively supplement a deficiency of a marine food component, for example, the fatty acid 20 : 5 $\omega$ 3 so that the food composition of the nauplii is adjusted to the food need of the larvae.

10 As a food additive fish oil or codliver oil may be used. The food additive is applied to the outer surface of particles, for example, of micronized rice chaff and rice starch grains. Rice starch grains are particularly suitable because these grains have a particle size distribution ( 2 15 to 10  $\mu\text{m}$  ) lying within the size of the alimentary mouth of the Artemia. Moreover, rice starch grains have satisfactory coating properties, that is to say, the additive satisfactorily adheres to the outer surface of the rice starch grain and disengages the surface only in the alimentary canal of the 20 Artemia. Since the specific weight of the particles is of the order of magnitude of the Artemia containing water, the particles coated with food additive remain floating in the water so that they are readily accessible to the Artemia.

Apart therefrom the particles intended for the Artemia 25 food may be particles of rice chaff, maize derivatives, manioc derivative and/or soya derivatives. In this way the deficient basic food is supplemented by the food additive and a carrier having no or only little nutritive value may be dispensed with.

The particles are coated by charging the food additive 30 in an organic solvent. The particles are subsequently dispersed in the solution containing the food additive, the additive adhering to the outer surface of the dispersed particles. Finally the organic solvent is evaporated, if necessary, at a reduced pressure to obtain the additive-coated particles.

35 The solvent is chosen so that the food additive is absorbed therein, the particles to be coated do not dissolve therein, the vapour tension of the organic solvent exceeds that of the additive, which permits of removing the solvent

by evaporation, and the polarity of the solvent is such that the particles can be dispersed in the solvent. In the case of micronized rice chaff grains the solvent is petroleum-ether and in the case of rice starch grains acetone or 5 chloroform may be used. Tests have shown that by rice starch grains coated with 10 % by weight of codliver oil the food composition of the offspring deposited by Artemia fed on these grains can be adjusted to the food need of the larvae feeding on these offsprings.

10 The nauplii 90 and cysts 14 respectively produced in accordance with the invention in the devices 1 and 33 and harvested by the harvesting means 11 are purified in the processing unit 41 and stored in a packing 67. The packing 67 containing nauplii 90 or cysts 14 is provided with a label 68.

15 This label has a specification indicating: from which Artemia strains originate the nauplii or cysts, for which larvae of fish or crustacea they are specifically suitable, in the case of cysts the development characteristics, the food composition and/or a quality mark indicating the concentration of toxic substances, if any.

On the label 68 may be mentioned guaranteed specifications relating to the packed nauplii or cysts because the nauplii or cysts are produced under controlled conditions in the devices 1 and 44 respectively, since in these devices 1  
25 and 44 the relevant parameters affecting the production and the quality of the animals are kept constant within accurately defined limits. The water quality can be kept constant because on the one hand fresh or purified water, both having a constant composition, is supplied to the flowing aqua-culture and on  
30 the other hand the residence time of the water in the flowing aqua-culture is so short that the excreta produced by the Artemia within said period does not significantly affect the water quality. Since the devices 1 and 44 are provided with heating means 80, insulated walls 15 and a cover 43, the  
35 selected temperature in the flowing aqua-culture can be maintained at an accurately defined value.

A flowing aqua-culture is distinguished from a batch

water and the water in the flowing aqua-culture is continuously refreshed. The water having passed through the flowing aqua-culture is continuously refreshed. The water having passed through the flowing aqua-culture may be evacuated or be partly 5 or wholly recycled. Before supplying the water again to the flowing aqua-culture the water to be recycled is purified in purifying units and freed of all or substantially all excreta produced by the Artemia during the passage through the flowing aqua-culture. A flowing aqua-culture with complete recirculation 10 of the flowing water is distinguished from a batch aqua-culture comprising a filter unit for separating out particulate excreta in that the recirculated water satisfies the same quality requirements applied to freshly supplied water so that the conditions under which the Artemia are kept in the flowing 15 aqua-culture are constantly the same. In the case of the batch aqua-culture comprising a filter unit for separating out particulate excreta, inter alia the water quality continues decreasing since the start of the aqua-culture despite the removal of the particulate excreta, whilst the conditions under which 20 the Artemia are kept in this batch culture are not constant, but are deteriorating from the instant of starting of the batch culture.

CLAIMS

1. A method of producing Artemia offspring (14, 90) in which the Artemia (49) depositing offspring (14, 90) are kept in an aqua-culture (2) and the deposited offspring (14, 90) is harvested, characterized in that the Artemia (49) in high density are kept in a flowing aqua-culture (2) under such selected conditions that the Artemia (49) deposit only one selected type (14, 90) of the two potential types of offspring (14, 90) i.e. nauplii (90) and cysts (14).

2. A method as claimed in Claim 1, characterized in that the Artemia (49) are kept in a density of at least 1000, preferably at least 10.000 and more, preferably at least 15.000 animals (49) per litre.

3. A method as claimed in Claim 1 or 2, characterized in that the deposited offspring (14, 90) is harvested from a water stream (46) leaving the flowing aqua-culture (2).

4. A method as claimed in one of the preceding Claims, characterized in that through a wedge strainer (26) arranged in the flowing aqua-culture (2) and retaining the Artemia (49) excreta (59) and water (46) containing produced offspring (14, 90) are conducted out of the flowing aqua-culture (2).

5. A method as claimed in Claim 4, characterized in that after harvesting the produced offspring (14, 90) at least a portion (46) of the water (46) conducted away as a carrier of offspring (14, 90) and excreta (59) is purified and recycled as a recycle stream (68).

6. A method as claimed in one of the preceding Claims,

characterized in that for producing Artemia cysts (14) the Artemia (49) are subjected to a treatment inducing a cyclic cyst production.

7. A method as claimed in Claim 6, characterized in that  
5 the treatment inducing the cyclic cyst production consists of  
a cyclic reduction of the oxygen concentration of the flowing  
aqua-culture (2).

8. A method as claimed in Claim 6 or 7, characterized in  
that the flowing aqua-culture (2) is flushed with water (25),  
10 the salinity of which lies between 15 and 90 ppt, preferably  
35 and 50 ppt, and more preferably with sea water (25).

9. A method as claimed in anyone of Claims 6 to 8,  
characterized in that the oxygen concentration in the flowing  
aqua-culture (2) is cyclically reduced in a period from 1 to  
15 6 hours for at the most 30 minutes, a minimum oxygen concen-  
tration being attained within 15 minutes, preferably within  
6 minutes, which is lower than or equal to 10 % of the  
saturation concentration of oxygen in the flowing aqua-culture  
(2).

20 10. A method as claimed in anyone of Claims 6 to 9,  
characterized in that the oxygen concentration is reduced  
by supplying nitrogen gas (64) to the flowing aqua-culture (2).

11. A device (1, 44) for producing Artemia offspring  
(14, 90) in aqua-culture (2) comprising a water basin (5),  
25 food supplementing means (6), sieving means (8) for removing  
excreta and produced offspring (14, 90) from the aqua-culture  
(2) whilst retaining the Artemia (49) producing the offspring  
(14, 90), aerating means (13) and harvesting means (11)  
characterized in that the device (1, 44) is provided with  
30 means (12, 53, 64, 67) for depositing only one selected type  
(14, 90) of the two potential types of offspring (14, 90),  
i.e. nauplii (90) and cysts (14) and in that the water basin  
(5) is provided with a water inlet (7) and a water outlet (4)  
for obtaining a flowing aqua-culture (2).

35 12. A device (44) as claimed in Claim 11, characterized  
in that the depositing means (12, 53, 64, 67) comprise indu-  
cing means (12, 53, 64, 67) for the cyclic reduction of the  
oxygen concentration in the flowing aqua-culture (2).

13. A device (1, 44) as claimed in Claim 11 or 12, characterized in that the harvesting means (11) comprise a wedge strainer (39).

14. A packing (67) of Artemia offspring (14, 90) as 5 claimed in anyone of Claims 1 to 5, i.e. Artemia nauplii (90) or cysts (14) produced as claimed in anyone of Claims 1 to 10, and provided with a label (68) indicating a specification of the packed offspring (14, 90).

15. A method of feeding Artemia (49) in which the 10 Artemia (49) are fed on a micronized product (7) characterized in that the Artemia (49) are fed on a carbohydrate- and/or protein-lean maize derivative (7), a soya derivative and/or a manioc derivative.

16. A method of feeding Artemia (49) in which Artemia 15 (49) are fed on a micronized product (7) characterized in that the Artemia (49) are fed on micronized chicken manure (7).

17. A method of preparing food (14, 90) for larvae of fish or crustacea, in which nauplii (90) released by the development of Artemia cysts (14) or deposited by ovoviparous reproduction are fed to the larvae characterized in that Artemia cysts (14) produced as claimed in anyone of Claims 1 to 10 in aqua-culture (2) or Artemia nauplii (90) produced as claimed in anyone of Claims 1 to 5 are used.

18. A method as claimed in Claim 17, characterized in 25 that at least one food additive of a type such is added to the aqua-culture (2) that the food composition of the nauplii (90) corresponds to the food need of the larvae.

19. A method of preparing food (14, 90) for larvae of fish or crustacea in which the food composition of nauplii 30 (90) obtained by Artemia (49) and serving as a food (14, 90) for larvae of fish or crustacea is adjusted to the food need of the larvae characterized in that at least one food additive is added to the Artemia (49) in the form of particles coated with the food additive.

35 20. A method as claimed in Claim 19, characterized in that the particles are coated with fish oil.

21. A method as claimed in Claim 20, characterized in that the particles are coated with codliver oil.

22. A method as claimed in anyone of Claims 19 to 21, characterized in that the particles are formed by food particles intended to feed the Artemia (49).

23. A method as claimed in anyone of Claims 19 to 21,  
5 characterized in that the particles are starch grains.

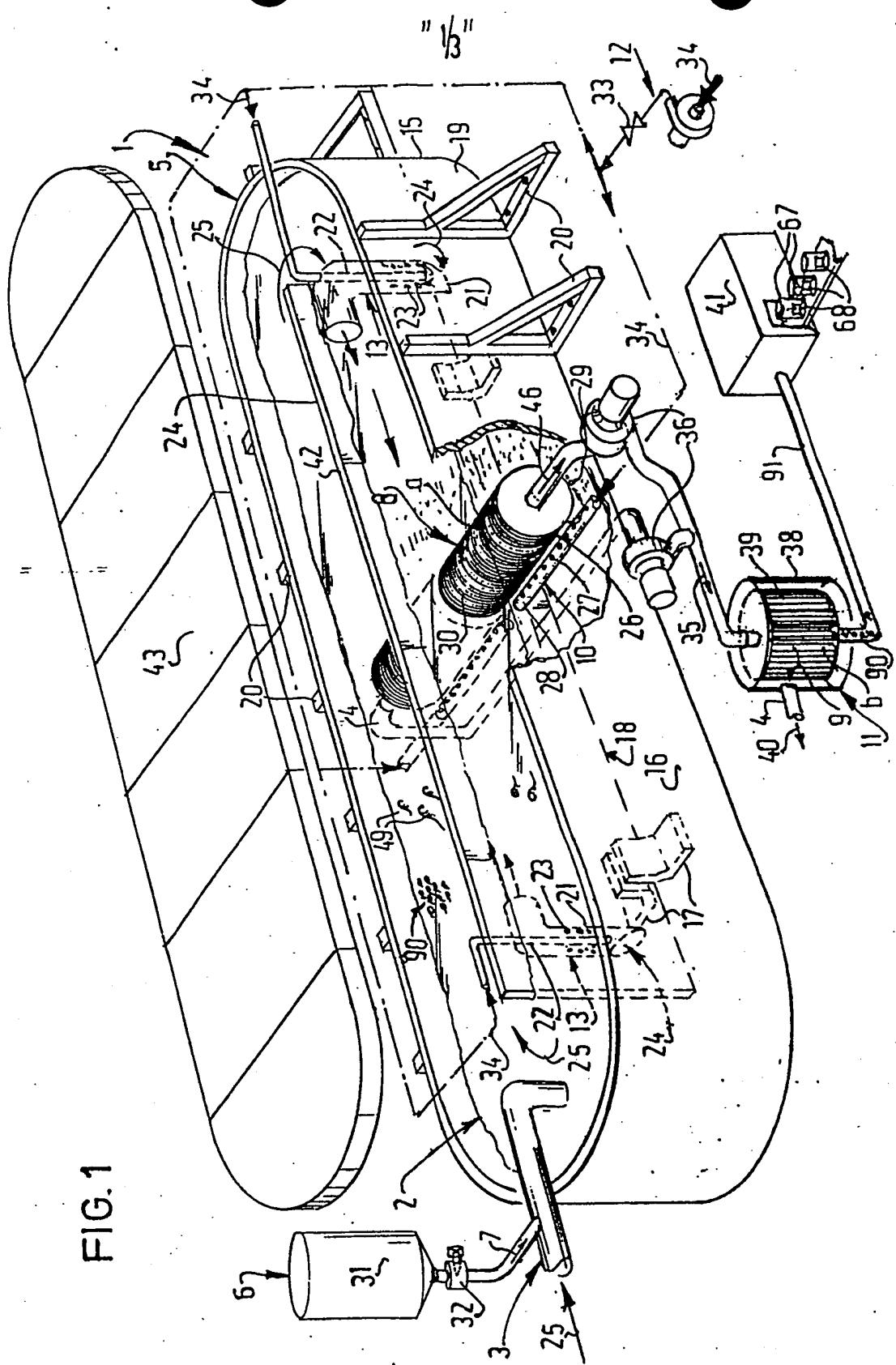
24. A method as claimed in anyone of Claims 19 to 23, characterized in that the food additive contains carotenoids.

25. A food containing particles coated with at least one food additive as claimed in anyone of Claims 29 to 24.

10 26. A method of preparing an animal food (49) characterized in that nauplii (90) produced by development of cysts (14) obtained as claimed in anyone of Claims 1 to 10 or nauplii (90) deposited by ovoviviparous reproduction as claimed in anyone of Claims 1 to 5 in an aqua-culture (2)  
15 comprising at least one wedge strainer (26) intended to conduct away water (52) containing excreta (59), whilst retaining the nauplii (90) are cultivated to obtain Artemia (49) and the cultivated Artemia (49) are subsequently harvested.

27. A method as claimed in Claim 26, characterized in  
20 that the nauplii (90) are grown in a flowing aqua-culture (2).

0104702



04702

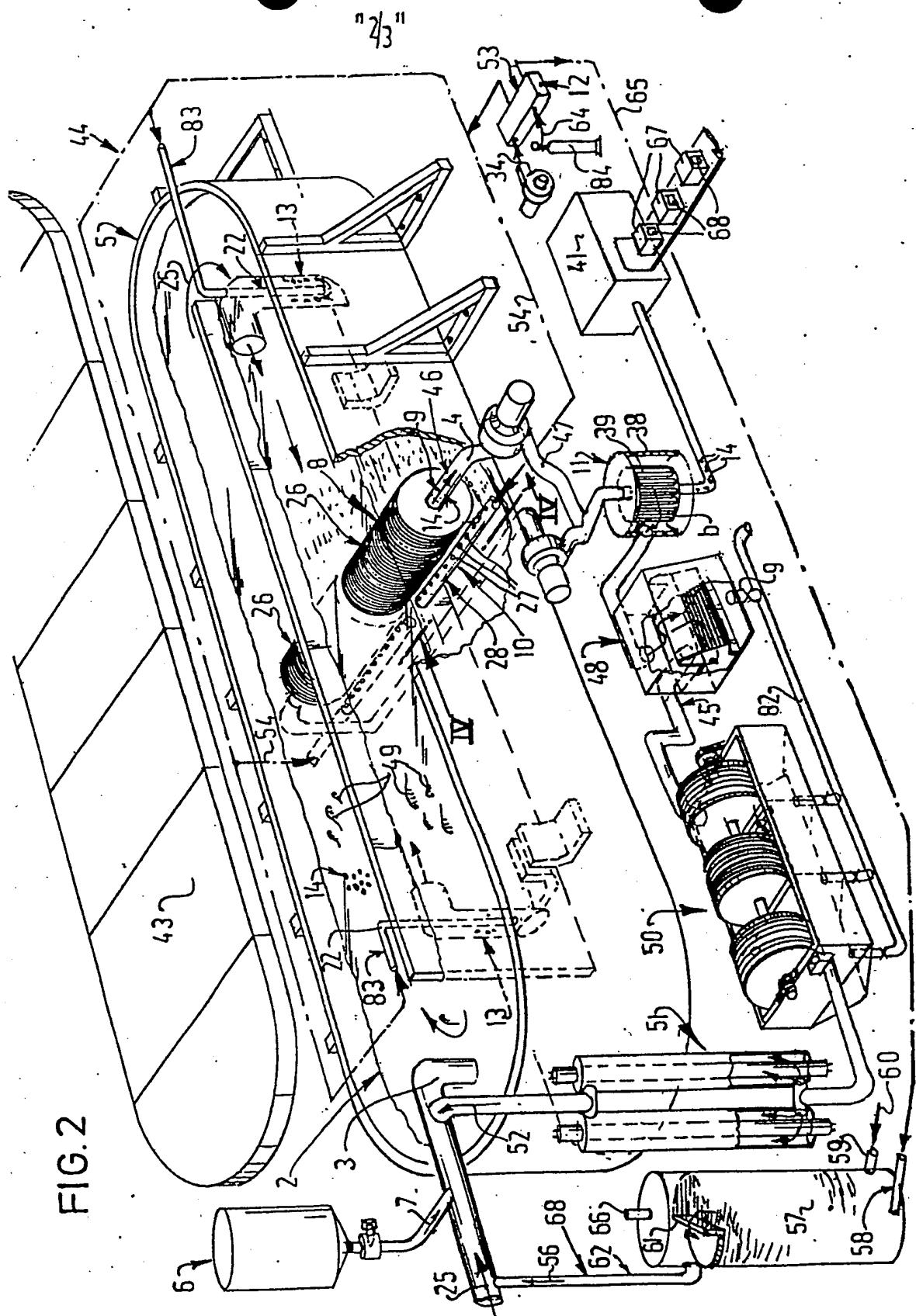


FIG. 2

0104702

"36"  
73

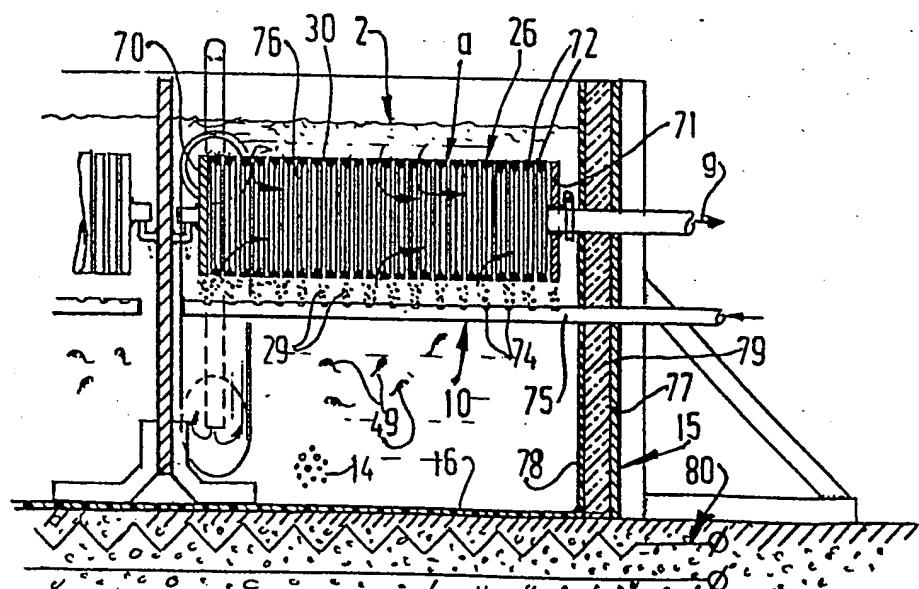
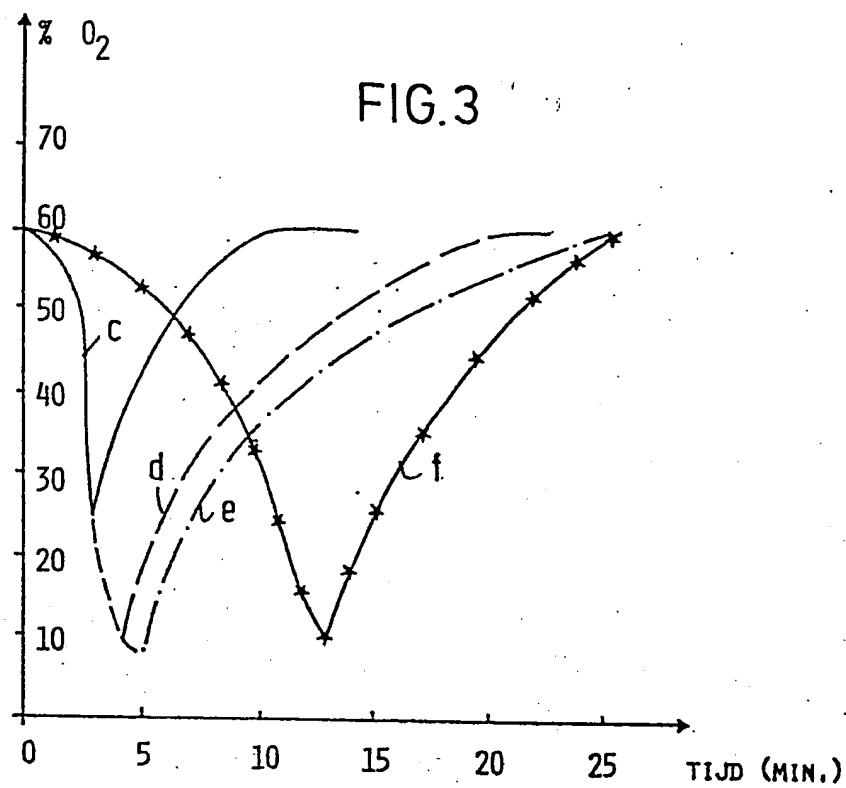


FIG.4



## EUROPEAN SEARCH REPORT

Application number

EP 83 20 1335

## DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. *)
			TECHNICAL FIELDS SEARCHED (Int. Cl. *)
A	GB-A-1 291 991 (SPENCER)		A 01 K 61/00 A 23 K 1/18
A	--- GB-A-1 592 085 (FISONS)		
A	--- US-A-4 163 064 (HILL)		
A	--- US-A-3 029 784 (ELBREDER)		
A	--- DE-B-1 047 521 (BLOMIN)		
	-----		A 01 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE	Date of completion of the search 30-12-1983	Examiner VILBIG K	
CATEGORY OF CITED DOCUMENTS	X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document	T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	